REMARKS

Upon entry of this Amendment, claims 13, 14, and 44-49 will be pending, of which claim 13 is independent. Claims 13 is amended to clarify that the minimal medium recited in steps b) and c) contains no methionine, S-adenosylmethionine, homocysteine, or cystathionine. Claim 48 is amended to correct a typographical error. Support for the claim amendments may be found at least at page 14, lines 23 to 32; page 23, lines 15 to 24; and page 45, lines 20 to 23 of the specification as filed, for example. Therefore, no new matter has been added.

Applicants thank the Examiner for withdrawing the objection to claim 42, and for withdrawing the rejection of claims 13, 14 and 38-41, and 43-49 as being unpatentable over Richaud *et al.* (*J. Biol. Chem.* 1993;268(36):26827-26835, hereinafter "Richaud") in view of Short *et al.* (U.S. Pat. App. Pub. No. 2005/0124010).

Rejections Under 35 U.S.C. § 103(a)

Claims 13, 14, and 44-49

Claims 13, 14, and 44-49 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Richaud in view of Schroder *et al.* (U.S. Pat. App. Pub. No. 2008/0118959, hereinafter "Schroder") and further in view of Thanbichler *et al.* (*J. Bacteriol.* 1999;181(2)662-665, hereinafter "Thanbichler").[1] Applicants respectfully traverse this rejection.

The Examiner bears the initial burden of factually supporting any *prima facie* conclusion of obviousness.[2] The key to supporting any rejection under 35 U.S.C. § 103 is the clear articulation of the reasons why the claimed invention would have been obvious. The Supreme Court recently observed that the analysis supporting a rejection under 35 U.S.C. § 103 "should be made explicit."[3] Moreover, if the Examiner fails to set forth a *prima facie* case of obviousness, Applicants are under no obligation to submit evidence of non-obviousness. Finally, "[i]n determining the differences between the prior art and the claims, the question under 35 U.S.C. § 103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious."[4]

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[1] *See* Non-Final Office Action, at 5 (17 Sept. 2009).

[2] *See, e.g.*, M.P.E.P. § 2142.

[3] *KSR Int'l Co. v. Teleflex, Inc.*, 550 U.S. 398, 418 (2007).

[4] M.P.E.P. § 2141.02(I) (citing *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530 (Fed. Cir. 1983); *Schenck v. Nortron Corp.*, 713 F.2d 782 (Fed. Cir. 1983)); *see also KSR*, 550 U.S. at 406.

Amended claim 13 is directed to, *inter alia*, growing a microorganism "on a minimal medium containing no methionine, S-adenosylmethionine, homocysteine, or cystathionine … in the presence of methylmercaptan," and to "selecting an evolved microorganism … wherein at least one protein has evolved in the methionine biosynthesis pathway allowing the modified microorganism to produce methionine."[5] Applicant submits that Richaud, Schroder, and Thanbichler—whether alone or in combination—fail to disclose at least the aforementioned features of independent claim 13.

In particular, Applicant submits that the primary citation to Richaud does not disclose cultivating the modified microorganism on a minimal medium containing no methionine, S-adenosylmethionine, homocysteine or cystathionine in the presence of methylmercaptan. Neither does Richaud teach the selection of an evolved microorganism wherein at least one protein has evolved in the methionine biosynthesis pathway allowing the modified microorganism to produce methionine. Accordingly, and without conceding the propriety of the asserted combination, the asserted combination of Richaud, Schroder, and Thanbichler is likewise deficient, even in view of the knowledge of one of ordinary skill in the art.

The Office action contends that the principal distinction between the claimed invention and the prior art is the disruption of the *met*E gene.[6] This contention is respectfully traversed.

Richaud teaches that—in *E. coli* strains that are auxotrophic for diaminopimelate—disruption of the *met*C gene and joint over-expression of the *met*B gene leads to the ability of these strains to grow on minimal medium. Since the *met*C gene—disrupted in Richaud—is involved in the methionine biosynthesis pathway, the modified strain disclosed by Richaud has impaired growth when grown on a minimal medium containing no methionine, S-adenosylmethionine, homocysteine or cystathionine. Importantly, Richaud does not teach disruption of the *met*E gene or culturing the modified microorganism obtained on a medium containing no methionine, S-adenosylmethionine, homocysteine or cystathionine in the presence of methylmercaptan. Richaud teaches expressly that homocysteine and cystathionine are added to the medium.[7] It is also clear that no methylmercaptan is added to the Richaud medium as a source of sulfur; instead, the Richaud medium contains

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[5] Claim 13, *supra*.

[6] *See* Non-Final Office Action, at 6 (17 Sept. 2009) ("Richaud et al. broadly teach a method for producing an evolved protein involved in methione biosynthesis pathway, comprising most of the steps set forth in the instant claims, except that rather than disrupting the *met*E gene, as in the instant claims, Richaud et al. disrupt the *met*C gene.").

[7] *See* Richaud, Tables III, V, and VI.

magnesium sulfate as its sulfur source.[8] Furthermore, the object of Richaud is not to restore a methionine biosynthetic pathway using methylmercaptan as a source of sulfur, but to allow the modified strains to use cysteine thioethers for building their cell wall (as indicated in the article's subtitle: "Recruitment of Cysteine Thioethers for Constructing the Cell Wall of *Escherichia coli*").[9]

Finally, Richaud also makes clear that the strain used was modified—before being cultured—with multiple copies of a plasmid bearing the *met*B gene, causing an overproduction of the cystathionine synthase produced by said gene. There is no such modification of the modified strain in the method of the invention. Thus, Richaud cannot reasonably be interpreted to disclose the aforementioned features of independent claim 13.

The secondary citation to Schroder relates to methionine biosynthesis via disruption of the *met*K gene, and is cited for its alleged disclosure of "deletion of *met*K gene," "culturing and fermentation of a methionine-producing microorganism with a reduced metK activity," and "growing the microorganisms in minimal medium with mercaptans as a sulfur source."[10] Applicant submits that Schroder does not add anything that would remedy the aforementioned deficiencies of Richaud.

In particular, Schroder fails to teach disruption of a gene involved in the methionine biosynthetic pathway to prepare a modified microorganism having an impaired growth when grown on a minimal medium containing no methionine, S-adenosylmethionine, homocysteine or cystathionine in the presence of methylmercaptan.

Indeed, the gene product of the *met*K gene is the S-adenosylmethionine synthase, which uses methionine as a substrate. By disrupting *met*K, methionine consumption is reduced and methionine production is increased. However—and contrary to the Office's comments—Schroder does not disclose disruption of the *met*K gene. Rather, Schroder discloses replacement with a mutant *met*K gene encoding an enzyme having reduced activity.[11] Schroder also makes clear that deleting the *met*K

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[8] *See id.*, at 26828 ("Growth of Bacterial Strains").

[9] *See id.*, at 26834 ("Discussion," last paragraph: "These strains can thus be viewed as having undergone an evolutionary commitment to use cysteine thioethers for building their cell wall.").

[10] *See* Non-Final Office Action, at 8 (17 Sept. 2009).

[11] *See* Schroder, at [0017] ("[T]his object is achieved by providing a method … which comprises the expression of a metK nucleotide sequence … the nucleotide sequence encoding an S-adenosylmethionine synthase mutant whose activity is modified, preferably reduced, over the wild-type enzyme.").

gene is not a solution to preventing the formation of S-adenosylmethionine because it is an essential gene.[12]

Finally, Schroder does not disclose culturing the modified microorganism for multiple generations under selection pressure with methylmercaptan.[13] In contrast, Shroder simply discloses culturing the microorganism on a culture medium in order to produce methionine.[14] Consequently, Schroder cannot reasonably be interpreted to disclose the aforementioned features of independent claim 13. Thus, Schroder also fails to add anything that would remedy the aforementioned deficiencies of Richaud; the combination of Richaud with Schroder would not lead one having ordinary skill in the relevant art to the claimed methods.

The tertiary citation to Thanbichler relates to the metabolism of methionine in *E. coli*, discussing genes involved in the metabolic pathway, and is cited for "teach[ing] the importance of *met*E and *meth* in the synthesis of methionine."[15] Applicant submits that Thanbichler does not add anything that would remedy the aforementioned deficiencies of Richaud and Schroder.

In particular, Thanbichler's study of "an *E. coli* mutant having a deletion of the *met*E gene for its involvement in alternative pathways of producing methionine" is irrelevant in relation to the claimed invention.[16] Deletion of the *met*E gene in Thanbichler was not done to allow the strain to evolve a metabolic pathway to compensate for the impaired growth. The Thanbichler strains were cultured on minimal growth medium supplemented with methionine or methyl-methionine.[17] Methionine is not produced in the Thanbichler experiments, but consumed to allow growth of the modified strain.

Moreover, Thanbichler nowhere indicates any culture of the modified microorganism on a minimal medium containing no methionine, S-adenosylmethionine, homocysteine or cystathionine

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[12] *See id.*, at [0015].

[13] *Cf.* claim 13, *supra* ("[T]he method comprising … b) culturing the modified microorganism obtained in step (a) on the said minimal medium containing no methionine, S-adenosylmethionine, homocysteine, or cystathionine for multiple generations, under selection pressure in the presence of methylmercaptan, allowing the modified microorganism to evolve a metabolic pathway to compensate for impaired growth….").

[14] *See, e.g.*, Schroder, at [0229].

[15] *See* Non-Final Office Action, at 8 (17 Sept. 2009).

[16] *Id.*

[17] *See, e.g.*, Thanbichler, at Figure 1.

for multiple generations under selection pressure with methylmercaptan to restore methionine production in the strain.[18]

Consequently, Thanbichler cannot reasonably be interpreted to disclose the aforementioned features of independent claim 13. Thus, Thanbichler also fails to add anything that would remedy the aforementioned deficiencies of Richaud and Schroder; the combination of Richaud with Schroder and Thanbichler would not lead one having ordinary skill in the relevant art to the claimed methods.

Claims 14 and 44-49 depend from independent claim 13, and so recite all of the limitations of claim 13. Accordingly, Applicant respectfully requests favorable reconsideration and withdrawal of the rejection of claims 13, 14, and 44-49 under 35 U.S.C. §103(a).

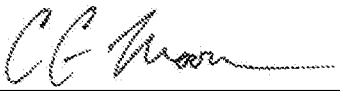
CONCLUSION

In view of the remarks above, Applicants respectfully submit that the stated grounds for rejection have been properly addressed and that all of the claims are patentable, and so request favorable action thereon. The Examiner is invited to contact the undersigned if any additional information is required.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 50-4254, under Attorney Docket No. 2912956-026000.

Respectfully submitted,

BAKER, DONELSON, BEARMAN, CALDWELL & BERKOWITZ, P.C.



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[18] *Cf.* claim 13, *supra* ("[T]he method comprising … b) culturing the modified microorganism obtained in step (a) on the said minimal medium containing no methionine, S-adenosylmethionine, homocysteine, or cystathionine for multiple generations, under selection pressure in the presence of methylmercaptan, allowing the modified microorganism to evolve a metabolic pathway to compensate for impaired growth….").